Is Hemolysis a Clinical Marker of *Propionibacterium acnes* Orthopedic Infection or a Phylogenetic Marker?

We read with great interest the study by Nodzo and colleagues in the May 2014 issue of *The American Journal of Orthopedics* on hemolysis as a clinical marker for *Propionibacterium acnes* orthopedic infection.[1] We agree with the authors that determining if a *P acnes* culture is a true infection or a contaminant remains a challenge. Although *P acnes* is described as a commensal bacterium with a low pathogenicity, its involvement has been reported in many clinical entities, especially device-related infections.[2] *P acnes* is usually the cause of delayed infections occurring 3 to 24 months or more after prosthesis placement. The rate of *P acnes* involvement, probably underestimated, is about 10%.[3] Although this bacterium was considered to be a contaminant, several virulence factors have been recently identified: putative hemolysins or cytotoxins (CAMP factors, hemolysin III) and enzymes putatively involved in degrading host tissue or molecules (GehA lipase, lysophospholipase, hyaluronate lyase, endoglycoceramidase, etc).[4]

Interestingly, Nodzo and colleagues revealed that 13 out of 22 *P acnes* strains were hemolytic and, among them, 10 were considered as definite infections, including 3 with only 1 positive sample. The authors could not identify a statistically significant trend, probably because their study was underpowered due to the size of this case series, as discussed by the authors. Nevertheless, the hemolytic activity of the strains was investigated in the 1980s by adding different concentrations of blood obtained from rabbits, sheep, or humans.[5] The hemolytic activity was recorded as *positive* when a clear, colorless zone around the colonies appeared or *weak* when slight and incomplete hemolysis under the colonies was found.[5] Depending on the erythrocyte origin, differences in the lytic action of hemolysin or cytotoxin may indicate the existence of various enzymes. These enzymes could have different levels of production and provide a distinct hemolytic profile. This hemolytic activity observation could also be correlated to the genetic background of the isolates.

In fact, from a genetic and epidemiological point of view, the sequence analysis of *recA* gene distinguished 2 distinct lineages of *P acnes*: types I and II.[4] The association of some strains with specific clinical presentations was also demonstrated. Later, McDowell and colleagues[6] reported 5 main phylogenetically distinct groups: IA, IB, IC, II, and III. It would have been interesting to know the phylogenetic groups of the strains tested in the study by Nodzo and coauthors, especially as Sampedro and colleagues[7] recently reported more phylogenetic groups IA and IB among *P acnes* strains involved in bone and joint infections. Both of these phylotypes are hemolytic, unlike phylotypes II and III, less often encountered in this clinical entity as reported recently.[8] We agree with the authors that hemolytic behavior may be one of the key factors in the variability in the pathogenicity of *P acnes* strains, suggesting that some strains could be more aggressive than others during deep infection. Another feature is likely the biofilm-production ability of the strains.[9,10]

According to our experience, the hemolysis behavior was slightly different depending on which blood agar plates were used to detect hemolytic properties. We have selected 8 isolates or reference ATCC strains from different phylotypes. Each isolate was seeded on 5 different blood agar plates with

Table. ***Propionibacterium acnes*** **Hemolysis Profile According to Origin and Phylotype**

Strain	Origin	Phylotype	Shaedler Agar With 5% Sheep Blood[a]	Brucella Agar With 5% Horse Blood[a]	Columbia Agar With 5% Horse Blood[b]	Mueller-Hinton Agar With 5% Sheep Blood[c]	Gardnerella Agar With 5% Human Blood[c]
ATCC 6919	Skin	IA	H > 10 mm	H < 5 mm	H > 10 mm	H > 10 mm	H > 10 mm
ATCC 11827	Skin	IA	H < 5 mm	NH	NH	NH	H > 10 mm
200-105[d]	Spine infection	IA	H < 5 mm	NH	NH	NH	NH
2002-78[d]	Spine infection	IB	H > 10 mm	H < 5 mm	H > 10 mm	H > 10 mm	NH
2002-8219[d]	Spine infection	IB	H > 10 mm	H < 5 mm	H < 5 mm	H > 10 mm	NH
2003-907[e]	Spine infection	IC	NH	NH	NH	NH	NH
2004-8460[f]	Spine infection	II	NH	NH	NH	NH	NH
2007-242	Health product[g]	III	NH	NH	NH	NH	NH

Abbreviations: H, hemolysis diameter observed around the colony; NH, nonhemolytic isolates.
[a]Becton, Dickinson and Co, Franklin Lakes, New Jersey.
[b]Oxoid Ltd, Basingstoke, United Kingdom.
[c]bioMérieux, Marcy l'Etoile, France.
[d]The same hemolysis profile was found with hip or knee prosthesis strain (n = 10).
[e]As the phylotype IC remains rare, only one strain has been tested.
[f]Seven strains involved in acne vulgaris (n = 2), spine infections (n = 2), or hip/knee prosthesis (n = 3) were nonhemolytic as well.
[g]Strain recovered from a preservative solution for organ transplant.

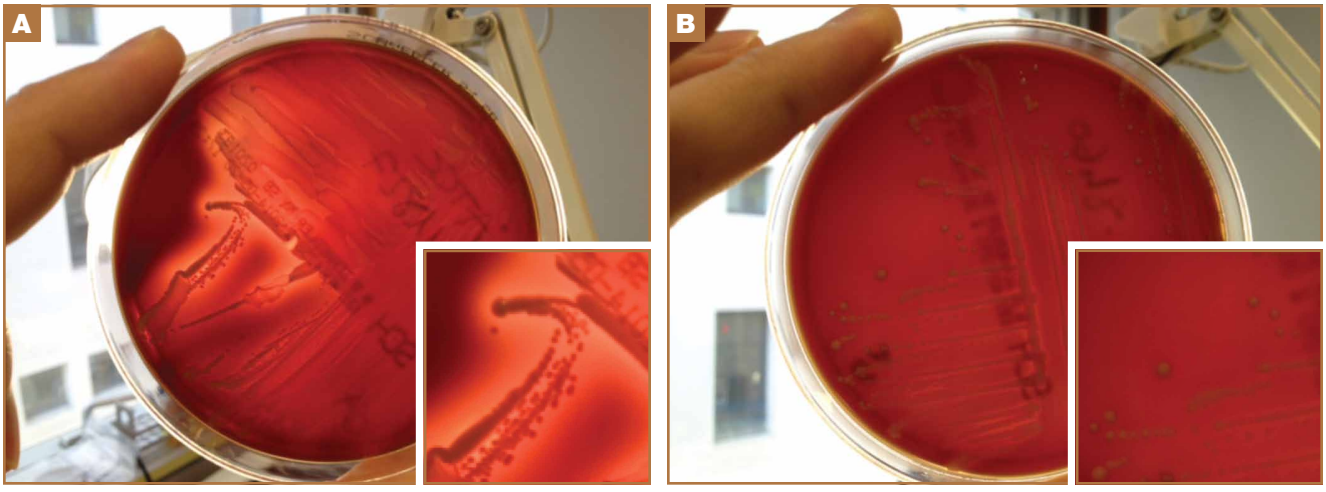

Figure. (A) Hemolytic and (B) nonhemolytic strains of *Propionibacterium acnes* belonging to genetic phylogroup (A) IA or IB and (B) II or III.

erythrocyte from various origins (**Table**). We can confirm that only strains belonging to IA and IB phylotypes were hemolytic, with different behavior as previously reported (**Figure**).[8] Similarly, within IA phylotype strains, the hemolytic property could be different suggesting a difference in the genetic background. However, as the genes encoding all 5 CAMP factors are present in all *P acnes* groups studied by Valanne and colleagues[11] (IA, IB, and II), observed differences reflected different levels of expression rather than missing genes. Moreover, when *camp2* or *camp4* genes were deleted, the Δ*camp2* but not the Δ*camp4* mutant exhibited reduced hemolytic activity with sheep erythrocytes, indicating that CAMP factor 2 seems to be the major active cohemolytic factor, but in an IA phylotype *P acnes* genetic background.[12]

To conclude, the link between hemolysis and *P acnes* deep infection remains controversial and complex. The phenotypic differences observed between strains from various types reflect deeper differences in their phylogeny. The hemolytic ability raises the possibility that strains may also display a specific behavior according to their type and variation in their expression of putative virulence factors, including hemolysin, cytotoxin, or lipase. Further studies are clearly needed to better understand the virulence and phylogeny of *P acnes* strains in order to distinguish contamination from bone infection.

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Authors' Response

Corvec and colleagues wrote an interesting summary and make excellent points about the role of hemolysis in *Propionibacterium acnes*. *P acnes* upper extremity infection has become an increasingly recognized problem, and determining whether a *P acnes* culture represents a true infection or a contaminant is still a challenge. We performed this study in hopes of finding an easily usable characteristic of *P acnes* that would assist the clinician in identifying *P acnes* strains as true infections rather than contaminants.

Certain pathogenic characteristics of *P acnes* have been identified, but the clinical implications of this bacterium are still being evaluated. We recognize that the hemolysis phenotype is a characteristic, and may not be the main pathogenic feature, of certain phylotypes of *P acnes*. It is possible the hemolytic strains in our study were from the IA and IB phylotypes, but, unfortunately, we did not specifically evaluate for phylogeny in our study. This would have correlated well with the work of Sampedro and colleagues,[1] which suggested most deep bone and joint infections occur with type IA and IB *P acnes* phylotypes. Although less common in orthopedic infections, the type II and III phylotypes of *P acnes* are also capable of causing deep infection, and may not cause a hemolytic reaction on blood agar, which may be why we had some patients classified as a definite infection that did not have a hemolytic strain of *P acnes*. It is also possible a hemolytic strain may truly be a contaminant, but we did not observe this in our small case series. A larger series may help elucidate this finding, but the majority of truly infected patients in our case series had a hemolytic *P acnes* phenotype.

The type of blood agar used could have also influenced our results, as noted in the **Table** in Corvec and colleagues' letter. We observed the most robust hemolysis on brucella blood agar, and limited hemolysis on CDC (Centers for Disease Control and Prevention) anaerobe blood agar; however, we did not evaluate multiple different blood agar preparations, which could have identified more hemolytic strains.

In our study, the presence of hemolysis was helpful in determining whether or not a true infection existed, but the absence of the hemolytic phenotype did not offer much additional information. The hemolytic phenotype may be a potential marker for those strains that are more aggressive and possibly represent the IA and IB phylotypes, which, as previously stated, are more commonly found in deep bone and joint infections.[1] Hemolysis may serve as a surrogate marker for determining these phylotypes since determining phylogeny in a hospital laboratory is burdensome and not possible in most institutions.

In summary, we agree the hemolytic phenotype is commonly observed in certain *P acnes* phylotypes, and that not all upper extremity orthopedic *P acnes* infections will have a hemolytic finding. The genetic differences in *P acnes* strains are complex, and finding a marker of truly pathogenic strains has yet to be established. Larger studies evaluating the clinical outcomes and laboratory findings of patients with and without hemolytic strains of *P acnes* and evaluating which blood agar is the best at identifying the hemolytic phenotype may be beneficial. Identifying or combining multiple clinical and microbe-specific characteristics may also help guide treatment recommendations when a positive *P acnes* culture is identified.

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